

Applicants: Kausik Si and Eric Kandel  
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Filed: March 21, 2007  
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**In the Specification:**

Please replace the paragraph starting on page 19, line 34 to page 20, line 2 with the following rewritten paragraph:

In an embodiment of either of the preceding two methods, the CPE comprises the sequence:

5'GGAATTCGGCACCATGTGCTTCTGTAAATAGTGTATTGTGTTTTTAATGTTGG  
ACTGGTTGGAATAAAGCTCTAGAGC-3' (SEQ ID NO:1).

Please replace the paragraph starting on page 26, line 3 to line 24 with the following rewritten paragraph:

Full length CPEB was cloned by degenerate PCR and 5' and 3' RACE (rapid amplification of cDNA ends). To make first strand cDNA total RNA was isolated either from *Aplysia* oocytes or pleural ganglia (as a source of sensory cells) using Trizol reagent (Life Technologies). 5ug of total RNA was reverse transcribed using Life Technologies first strand cDNA synthesis kit. For degenerate PCR cDNA prepared from *Aplysia* oocytes was amplified by Taq DNA polymerase (Roche) using 100 pMol of each primer in the PCR cycle: 94°C 1 min, 42°C 1min, 72°C 1 min -30 cycles. A 500 bp cDNA fragment was cloned. Two degenerate primers CPEB1-5' GCGG-AATTCGTNSARGTNATHCCNTGG-3' (SEQ ID NO:2) and CPEB2- 5' GCGGGATCCT-GNTGCCANTSCCARCA-3' (SEQ ID NO:3) were designed from the conserved C-terminal RNA binding domain of CPEB from several other species. Using the sequence information from the 500 bp cDNA fragments,

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5' and 3' Rapid Amplification of cDNA Ends (RACE) were carried out on CNS and ovatestis mRNA (Clontech). The 5' RACE primers 5'-CACTGTCTTGTTCGACTCCAG-3' (SEQ ID NO:4) and 5'- AACACATGGTTACT-GTCCGC-3' (SEQ ID NO:5) and 3' RACE primer 5'- CATGAAAGCCGTGCAAGCTGCATT-3' (SEQ ID NO:6) were used. The protein encoded by this cDNA fragment had ~80% homology to the RNA binding domain of CPEB of other species.

Please replace the paragraph starting on page 26, line 26 to page 27, line 9 with the following rewritten paragraph:

PolyA mRNA's of different developmental stages of *Drosophila* were purchased from Clontech. To obtain adult head and body mRNA 50 *Drosophila* heads were manually dissected and total mRNA was prepared using the TriZol method. The first strand cDNA was made from 1ug of PolyA mRNA or 5ug of total mRNA. The following primer pairs were used for the PCR: CG5735RA -5' primer - CGGGATCCATGTACAACAAATTTGTTA (SEQ ID NO:7) and 3' primer - TCCCCGCGGCG-ATCCTCCGCCTCCTC (SEQ ID NO:8); CG5735RB-D- 5' primer- ATGGACTCGCTCAAGTTACCA (SEQ ID NO:9) and 3' primer - TCCCCGCGGCG-ATCCTCCGCCTCCTC (SEQ ID NO:8); Orb- 5' primer - CGCG-ATGCCTGATTGATTGTTGAA (SEQ ID NO:10) and 3'- primer- TGTGCGTTATTTTATCGTTTAGTG (SEQ ID NO:11); rp49- 5' primer- GACTTCATCCGCCACCAAGTCG (SEQ ID NO:12) AND 3' primer-CACCAGGAACT-TCTTGAATCCG (SEQ ID NO:13). To clone the CG5735RA open reading frame, first strand cDNA made from adult head mRNA was PCRed with the 5' primer- CGGGATCCATGTACAAC-AAATTTGTTA (SEQ ID NO:7) and 3' primer

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- CCCTCGAGAAGCTTTTAAACACCAGCGAAAGG-GGAC (SEQ ID NO:14) and cloned into the BamHI/XhoI site of pRSETA (for His<sub>6</sub>-tag) or into the same sties of pKS(+). For expression in TnT lysate the pKS(+)-CG5735RA was transcribed with T3 RNA polymerase.

Please replace the paragraph starting on page 27, line 12 to line 19 with the following rewritten paragraph:

To clone DroGEF single stranded cDNA made from *Drosophila* brain mRNA was PCR amplified using 5' primer CGGGATCCATGCT-GGACAGCAACAACAG (SEQ ID NO:15) and 3' primer GACTAGTCTAGAATAGATTAGCAAAG-AAATC (SEQ ID NO:16). The PCR product was cloned into the pCRTOP0II vector (invitrogen) and digested with BamH1. The ~1.8 kb fragment was cloned into the same site of pGEX2T and a clone in which the insert was in correct orientation was subsequently used to transform *E.coli* BL21(DE3).

Please replace the paragraph starting on page 27, line 21 to page 28, line 11 with the following rewritten paragraph:

The neuronal actin 3'UTR was obtained by PCR using the 5' primer 5'-GGGAATTCGTCTGGAGCCACCAACAC-3' (SEQ ID NO:17) and 3' primer 5'-CGGATCCAT-TTATTAACATTGTATAAAAAATACAGTTG AAC-3' (SEQ ID NO:18). To mutate the CPE element the 3'primer was changed to 5'- CGGATCCATTTATTAACA-TTGATATGGGAAATACAGT-TGAAC-3' (SEQ ID NO:19). The N-actin 3UTR's were cloned into the pCR2.1 vector (Invitrogen). To create the Luciferase-SV403' UTR construct, pGL3-Basic

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(Promega) was digested with HindIII-BamHI and ligated into the same site of the vector pKSII. To make the Luciferase-Actin 3' UTR constructs, the pKSII-Luciferase-SV403UTR construct was digested with ApaI-XbaI. The Luciferase ORF was isolated and cloned into the same site of pCR2.1-Actin 3'UTR to create the Luciferase-Actin 3'UTR construct. To make <sup>7</sup>Methyl Guanosine capped mRNA the pKSII-luciferase-SV40 was linearized with BamHI and transcribed with T3 RNA polymerase using mMEASGE mMACHINE (Ambion). The pCR2.1 luciferase-Actin 3'UTR was transcribed with T7 RNA polymerase following digestion with BamHI. The mRNA was phenol-chloroform extracted, precipitated with ethanol and resuspended in nuclease free water. *X. laevis* oocytes were isolated and injected as described previously. Five oocytes were homogenized in 150 ul of luciferase cell lysis buffer (Roche). The total homogenate was centrifuged at 14,000 rpm in a micro centrifuge for 10 min and 10 ul of the clear lysates was assayed. The reaction was started by injecting 100 ul of the luciferase substrate (Roche).

Please replace the paragraph starting on page 30, line 27 to line 30 with the following rewritten paragraph:

CPEBQ, consisting of the N-terminal 480 nucleotides of ApCPEB, was amplified by PCR from the full length *Aplysia* CPEB cDNA using the following primers (written 5' to 3'):  
5': CGGGATCCATGCAAGCCATGGCCGT (SEQ ID NO:20); 3':  
TCCCCGCGGTGGACCAGGCGTGTA (SEQ ID NO:21).

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Please replace the paragraph starting on page 31, line 5 to line 9 with the following rewritten paragraph:

pGEM-CPE, consisting of 78 nucleotides of the cyclin B1 3'UTR, was prepared by hybridizing the oligo, 5'GGAATTCGGCACCATGTGCTTCTGTAAATAGTGTATTGTGTTTTTAATGTTGG ACTGGTTGGAATAAAGCTCTAGAGC-3' (SEQ ID NO:1), with its antisense oligo and cloning into the EcoRI/XbaI site of pGEM7Z(f+).

Please replace the paragraph starting on page 31, line 31 to page 32, line 2 with the following rewritten paragraph:

Two rabbit antisera were raised, one against the 17 amino acid C-terminal peptide <sup>644</sup>LCNSHQGNFYFCRDLLCF<sup>660</sup> (CPEB77) (SEQ ID NO:22) and one against the purified recombinant His<sub>6</sub>-CPEB (533). The CPEB77 antibody was affinity purified in a peptide column (Babco).

Please replace the paragraph starting on page 36, line 4 to line 19 with the following rewritten paragraph:

The polyA test (PAT) to measure the length of the polyA tail of CPEB mRNA was essentially done as described by Salles and Strickland (1999). Total RNA from the plural ganglia was digested with DNaseI and phenol: chloroform extracted. 3 ug of total RNA was reverse transcribed with 200 ng of AdT primer (5'-GCGAGCTCCGCGCCGCGTTTTTTTTTTTTT-3') (SEQ ID NO:23) using AMV reverse transcriptase. 2 ul

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of the cDNA was amplified with Taq polymerase using AdT and N-actin primer 5'-GGGAATTCGTCTGGAGCCACCAACAC-3' (SEQ ID NO:17) in the PCR cycle: 94°C 30 sec, 60°C 1 min and 72°C 1 min, 5 sec/cycle for 30 cycles. To check the amount of sensorin, Bmp1 and N-actin, the following primer pairs were used; sensorin- 5'-AACAGAAACAGTC-TTTCCCCC-3' (SEQ ID NO:24) and 5'-TCTTGACTCACCAACTGCC-3' (SEQ ID NO:25), Bmp1-5'-ATCTATCGCCTATT-ATTATCACCA-3' (SEQ ID NO:26) and 5'-ATCCCATGCATTTGTTTGT-3' (SEQ ID NO:27), N-actin 5'-CCCATCCAT-TGTCCACA-3' (SEQ ID NO:28) and 5'-TTTGAGCATTCTGGCTTC-3' (SEQ ID NO:29).

Please replace the paragraph starting on page 37, line 10 to line 22 with the following rewritten paragraph:

The antisense oligo 5'-AAACAGAGCAGGTC-CCGGCAGAAATAGT-3' (SEQ ID NO:30) was pressure injected into the cell. The oligo-peptide conjugate was purchased from Alta Biosciences (Birmingham, UK) and consisted of the TAT sequence (with a four glycine linker at amino terminal connecting it to a FITC) linked, via a disulfide bond, to the oligo (disulfide to the 5' end of oligo). The oligo was dissolved in water and diluted to a concentration of 100 uM in a buffer containing (as a 10X solution): 23.5 g NaCl, 0.744 g KCl, 7.14 g Dextrose, 0.192 g NaHCO<sub>3</sub> per 100 ml of solution, pH 7.6. About 0.5-1 ul of solution was perfused onto the branches for about 30 min 4hrs prior to bath applications of 5 pulses of serotonin (all cells had prior been tested for basal EPSPs).

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**Sequence Listing**

Please insert into the application the Sequence Listing attached hereto as **Exhibit B**.